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Combined Toxicity of 2,4-Dichlorophenoxyacetic Acid and Its Metabolites 2,4-Dichlorophenol (2,4-DCP) on Two Nontarget Organisms

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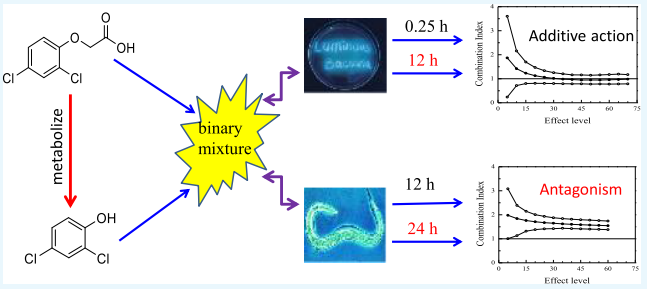
Supporting Information

ABSTRACT: 2,4-Dichlorophenoxyacetic acid (2,4-D), a phenoxyalkanoic acid herbicide, is among the most widely distributed pollutants in the environment. 2,4-Dichlorophenol (2,4-DCP), as the main metabolite of 2,4-D, always accompanies 2,4-D. In this paper, we did research on the combined toxicities of 2,4-D and 2,4-DCP to *Vibrio qinghaiensis* sp.-Q67 (Q67) and *Caenorhabditis elegans*. It was found that the toxicity of 2,4-DCP is more severe than that of its parent 2,4-D at any concentration levels whether to Q67 or to *C. elegans*. Furthermore, 2,4-DCP to Q67 has the time-dependent toxicity. The toxicity of the mixture of 2,4-D and 2,4-DCP to Q67 is increasing with the exposure time, but that to *C. elegans* does not change over time. There is a good linear relationship between the pEC_{50}/pLC_{50} value of binary mixture ray of 2,4-D and 2,4-DCP and the mixture ratio of 2,4-DCP, which implies the predictability of mixture toxicity of 2,4-D and 2,4-DCP. The toxicological interactions of the binary mixtures to Q67 are basically additive actions whether at 0.25 or at 12 h. However, most mixtures have antagonistic interactions against *C. elegans*.



INTRODUCTION

2,4-Dichlorophenoxyacetic acid (2,4-D), a phenoxyalkanoic acid herbicide, was first synthesized in 1941 and commercially marketed in the United States in 1944.[1] 2,4-D has been widely used for controlling many types of broad leaf weeds, grasses, and other monocots.[2] 2,4-D is often detected in water, soil, and air. Recent study has shown that 2,4-D was the most frequently detected herbicide in the suburban surface waters in the United States during 1999−2010, with the highest concentration of 0.46 μg/L.[3] It was reported that the concentrations of 2,4-D in soil were 1.8 and 4.6 ng/g at the 95th percentiles for North Carolina and Ohio State of United States, respectively.[4] In the 95th percentiles for North Carolina, the air concentration of 2,4-D was up to 1.7 ng/m.[3,4] Numerous studies have proven that 2,4-D may be an environmental hazard.[5,6] It can reduce growth rates, induce reproductive problems, or could cause death of nontarget species including plants and animals.[7−9]

2,4-D is moderately persistent in the environment and the half-life of it is about 15−312 days. In both anaerobic and aerobic conditions, the major metabolite of 2,4-D is 2,4-dichlorophenol (2,4-DCP).[10] 2,4-DCP is also frequently detected in the environment. 2,4-DCP was detected in more than half of China's surface water samples with the highest concentration of 19 960 ng/L.[11] In the surface water of the Taihu Lake, the maximum concentration of 2,4-DCP was 143 ng/L.[12] 2,4-DCP is characterized by high toxicity and persistence in the environment.[13−15] 2,4-DCP at low concentrations resulted in developmental disorders in zebrafish embryos.[14] In rats, 2,4-DCP had a weak reproductive toxicity. Therefore, it was listed as priority pollutants by China as well as the United States.[16]

Metabolism of a pesticide is not necessarily a process of detoxification. On the one hand, the toxicity of a metabolite is not necessarily less than that of its parent compound. It was shown that the acute toxicity induced by imidacloprid was LD_{50} = 57 μg/kg and the LD_{50}s values of the metabolites were 258 μg/kg (5-hydroxyimidacloprid) and 28 μg/kg (olefin), which suggested that the metabolite may be more toxic (olefin) or less toxic (5-hydroxyimidacloprid) than its parent.[17] Some parent herbicides such as 2-methyl-4-chlorophenoxyacetic acid, mecoprop, 2,4-D, and dichlorprop displayed a less inhibitory activity to acetylcholinesterase than their metabolites.[18] On the other hand, pesticides and their metabolites are

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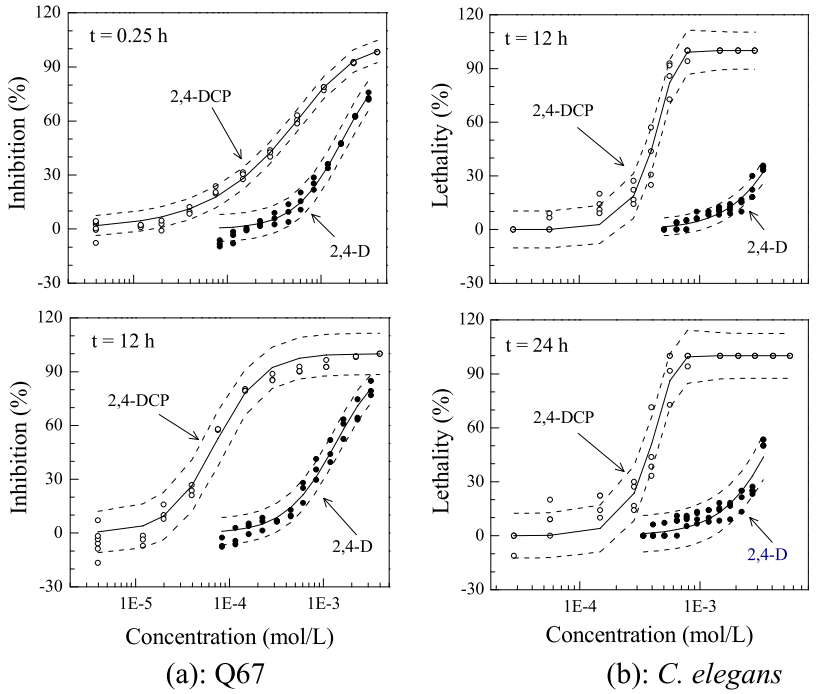

Figure 1. CRCs of 2,4-D and 2,4-DCP at different exposure times to Q67 (a) and *C. elegans* (b), where ● and ○ refer to the experimental scatters, solid curve (—) refers to the fitted curve, and dashed curves (---) refer to the 95% OCIs.

Table 1. Regression Coefficients (α and β), Fitting Statistics (R^2 and RMSE), and pEC_{50} (to Q67)/pLC_{50} (to *C. elegans*) of Chemicals and Mixture Rays

organism (time)	chemical/rays no.	function	α	β	RMSE	R^2	pEC_{50}/pLC_{50}
Q67 (0.25 h)	2,4-D	Logit	10.74	3.88	0.0305	0.9898	2.768
	2,4-DCP	Weibull	5.75	1.79	0.0225	0.9965	3.417
	R1	Logit	10.07	3.70	0.0262	0.9855	2.722
	R2	Weibull	7.10	2.69	0.0196	0.9914	2.776
	R3	Weibull	6.77	2.32	0.0244	0.9921	3.076
	R4	Logit	9.01	2.91	0.0223	0.9903	3.096
	R5	Weibull	6.45	2.10	0.0237	0.9916	3.246
Q67 (12 h)	2,4-D	Logit	10.86	3.78	0.0312	0.9900	2.873
	2,4-DCP	Logit	16.98	4.09	0.0472	0.9920	4.152
	R1	Logit	10.44	3.25	0.0431	0.9699	3.212
	R2	Logit	9.80	2.87	0.0321	0.9810	3.415
	R3	Logit	12.10	3.40	0.0461	0.9775	3.559
	R4	Logit	13.05	3.47	0.0542	0.9619	3.761
	R5	Logit	14.13	3.59	0.0373	0.9883	3.936
C. elegans (12 h)	2,4-D	Weibull	8.62	3.87	0.0196	0.9604	2.322
	2,4-DCP	Weibull	23.32	7.01	0.0413	0.9929	3.379
	R1	Weibull	14.50	6.11	0.0288	0.9731	2.433
	R2	Weibull	15.29	6.42	0.0275	0.9448	2.439
	R3	Weibull	14.12	6.00	0.0294	0.9586	2.414
	R4	Weibull	19.34	7.50	0.0560	0.9708	2.628
	R5	Weibull	28.14	9.97	0.0328	0.9933	2.859
C. elegans (24 h)	2,4-D	Weibull	9.09	3.90	0.0410	0.9105	2.425
	2,4-DCP	Weibull	22.23	6.63	0.0509	0.9881	3.408
	R1	Weibull	13.57	5.66	0.0303	0.9736	2.462
	R2	Weibull	13.15	5.51	0.0360	0.9186	2.453
	R3	Weibull	13.01	5.46	0.0261	0.9744	2.450
	R4	Weibull	15.60	6.00	0.0705	0.9516	2.661
	R5	Weibull	27.29	9.59	0.0433	0.9887	2.884

often detected to coexist in the same environmental medium[19] to form various mixtures. The combined toxicities of the mixtures of parent pesticide and its metabolites may be additive, synergistic, or antagonistic.[20] In other words, the toxicities of some mixtures are predictable (for additive), whereas those of others are unpredictable (for synergistic and

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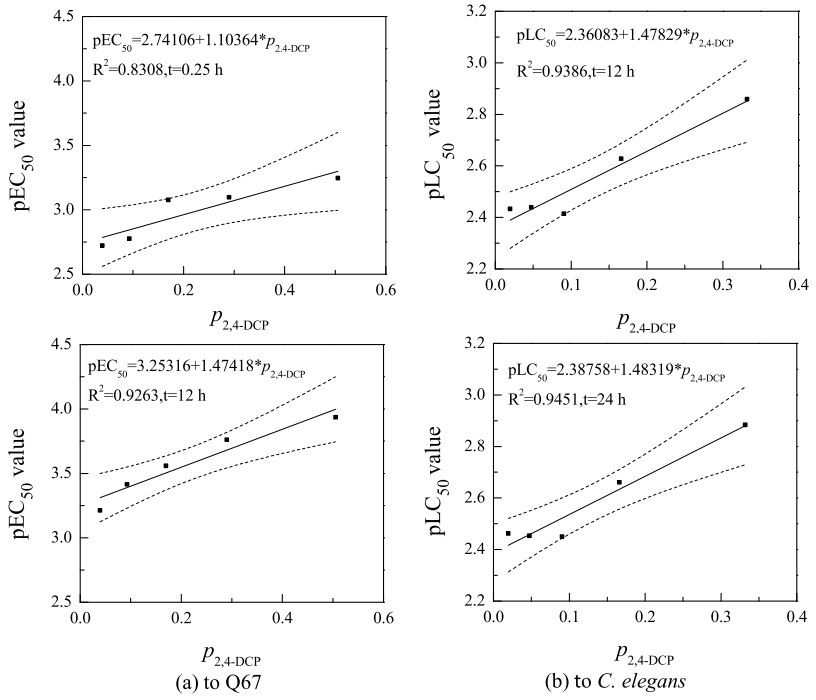

Figure 2. Linear relationship between $p_{2,4\text{-DCP}}$ and pEC_{50} [to Q67 (a)] or pLC_{50} [to *C. elegans* (b)] [solid curve (—) refers to the fitted curve and dashed curves (---) to the 95% confidence intervals].

antagonistic). For example, the mixtures of diuron and its metabolites (1-(3,4-dichlorophenyl)-3-methyl urea and 3,4-dichloroaniline) resulted in synergism in almost all cases, for both species of phytoplankton,[21] which implied that it is necessary to carefully examine the mixture toxicity to reveal the possible risks of the mixture to organisms. Cáceres et al.,[22] who were the first to study the interactive effect of chlorpyrifos and its metabolite 3,5,6-trichloropyridinol to a cladoceran, also suggested that the joint action of pesticides and their metabolites should be considered in the development of water quality guidelines.

The main purpose of this paper is to (1) determine the toxicities of the pesticide (2,4-D) and its metabolite (2,4-DCP) as well as their various mixtures to two nontarget organisms, the aquatic organism *Vibrio qinghaiensis* sp.-Q67 (Q67), a freshwater luminescent bacterium widely used in the determination of toxicity of chemical pollutants,[23] and the terrestrial organism *Caenorhabditis elegans* having excellent characteristics such as well-characterized genome, ease of maintenance, short and prolific life cycle, and small body size, making it a good model for toxicological analysis;[24,25] (2) evaluate the toxicity of 2,4-D and 2,4-DCP to different tested organisms; and (3) reveal whether the mixture toxicity can be predicted by the analysis of toxicological interaction to provide the basic data for the possible risk assessment caused by pesticide mixtures.

RESULTS

Toxicity of a Single Chemical. The concentration–response curves (CRCs) of pesticide (2,4-D) and its metabolite (2,4-DCP) to Q67 are shown in Figure 1a. The fitted regression coefficients (α and β), statistics [the fitted coefficient of determination (R^2) and root mean square error (RMSE)], and pEC_{50} (for Q67) are given in Table 1. Figure 1a shows that the four CRCs of 2,4-D and 2,4-DCP to Q67 are all classical monotonic S-shaped curves. The CRCs are different from each other and the toxicity of 2,4-DCP is always more severe than that of 2,4-D at the same exposure time and at any concentration level because the CRC of 2,4-DCP is located on the left side of the CRC of 2,4-D. For example, taking pEC_{50} as a toxicity index, the toxicity of 2,4-D (pEC_{50} = 2.768 and 2.873) is less than that of 2,4-DCP (3.417 and 4.152) (see Table 1) at two exposure times (0.25 and 12 h).

If the CRCs of the same chemical at two exposure times are plotted (see Figure S1a of the Supporting Information), it can be concluded that the two CRCs of 2,4-D to Q67 at 0.25 and 12 h are basically overlapping each other, whereas the CRCs of 2,4-DCP are different and the CRC at 12 h is located at the left side of that at 0.25 h, which implies that the inhibition toxicities (I) of 2,4-DCP to Q67 at 12 h are more severe than those at 0.25 h at many concentration levels. That is, different from 2,4-D, 2,4-DCP to Q67 has a time-dependent toxicity.

Figure 1b shows that the CRC profiles of pesticide 2,4-D and its metabolite 2,4-DCP to *C. elegans* are also classical S-shaped curves, and at the same exposure time, the lethal toxicities (L) of 2,4-DCP to *C. elegans* are more severe than those of 2,4-D at any concentration level because the CRCs of 2,4-DCP are located on the left side of those of 2,4-D. For example, taking the pLC_{50} to *C. elegans* as a toxicity index, the pLC_{50} values of 2,4-DCP are 3.379 and 3.408 at 12 and 24 h, respectively, which are larger than those (2.322 and 2.425) of 2,4-D (see Table 1).

If the CRCs of the same chemical at two exposure times are plotted (see Figure S1b of the Supporting Information), it can be concluded that two CRCs of 2,4-DCP or 2,4-D at two exposure times are basically overlapping each other, which implies that 2,4-DCP and 2,4-D have no time dependence to *C. elegans*.

Mixture Toxicity of 2,4-D and 2,4-DCP. The 10 CRCs of 5 mixture rays (R1, R2, R3, R4, and R5) of 2,4-D and 2,4-DCP to Q67 can be well described by Weibull or Logit functions

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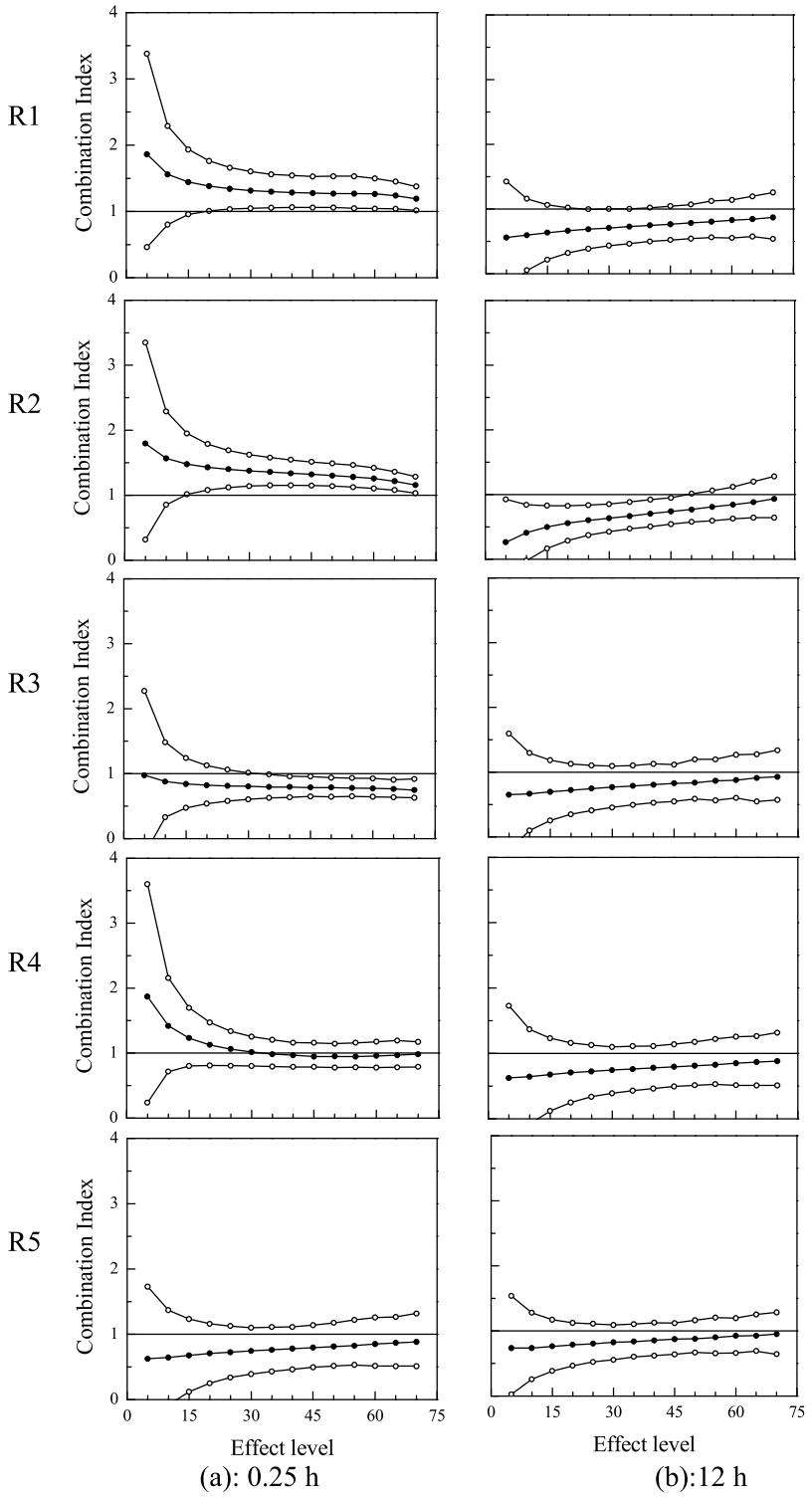

Figure 3. Plots of CI vs effect level of Q67 at two exposure times [0.25 h (a) or 12 h (b)] where the black dots (●) refer to CI and the hollow dots (○) to its 95% OCIs.

(see Figure S2a of the Supporting Information). The fitted regression coefficients (α and β), statistics [the fitted coefficient of determination (R^2) and RMSE], and pEC_{50} are given in Table 1. Figure S2a of the Supporting Information shows that the toxicity of mixture rays to Q67 is increasing with the exposure time. According to the pEC_{50} values of the mixture rays, the toxicity of the mixture ray increases monotonically with the mixture ratio of 2,4-DCP at the same exposure time. For example, the pEC_{50} values of R1, R2, R3, R4, and R5 to Q67 are 2.722, 2.776, 3.076, 3.096, and 3.246 at 0.25 h and 3.212, 3.415, 3.559, 3.761, and 3.936 at 12 h, respectively (see Table 1). The pEC_{50}s of mixture rays is well correlated with the mixture ratios of 2,4-DCP ($p_{2,4\text{-DCP}}$) (see Figure 2a).

The 10 CRCs of 5 mixture rays (R1, R2, R3, R4, and R5) of 2,4-D and 2,4-DCP to *C. elegans* can be fitted by Weibull

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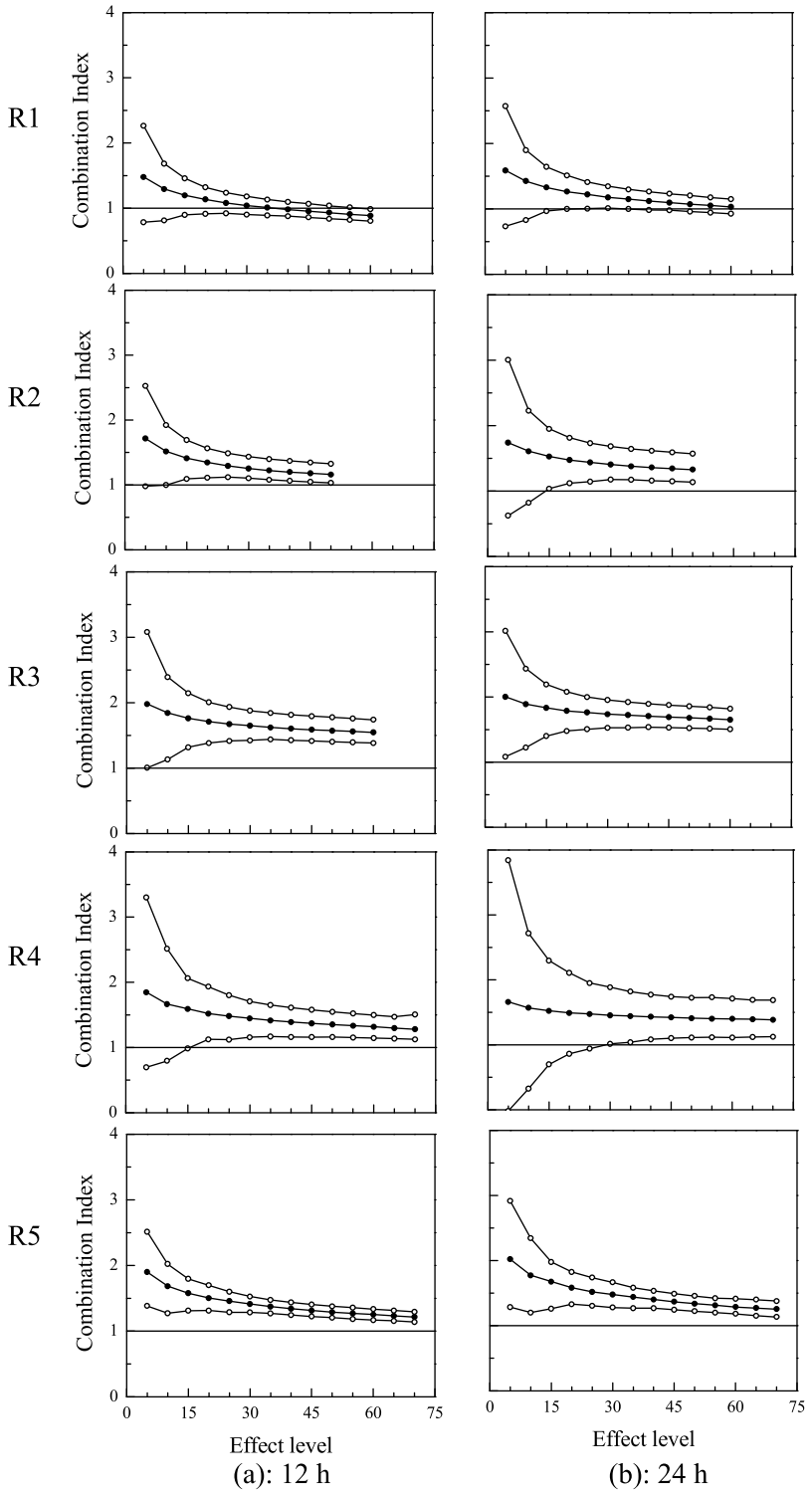

Figure 4. Plots of CI vs effect level of *C. elegans* at two exposure times [12 h (a) or 24 h (b)] where black dots (●) refer to CI and hollow dots (○) to its 95% OCIs.

function (see Figure S2b of the Supporting Information). Figure S2b shows that the toxicity of mixture rays to *C. elegans* is not time dependent. According to the pLC_{50} values of the mixture rays, the toxicity of the mixture ray monotonically increases with the mixture ratio of 2,4-DCP at the same exposure time. For example, the pEC_{50} values of R1, R2, R3, R4, and R5 to *C. elegans* are 2.433, 2.439, 2.414, 2.628, and 2.859 at 12 h and 2.462, 2.453, 2.450, 2.661, and 2.884 at 24 h, respectively (see Table 1).

The pLC_{50}s of mixture rays are well correlated with the mixture ratios of 2,4-DCP ($p_{2,4\text{-DCP}}$) (Figure 2b).

Toxicological Interactions in Mixtures. Plots of combination indices at a specific effect (x %) (CI_x) with 95% observation-based confidence intervals (OCIs) of five mixture rays to Q67 versus the percent effects (x %) are shown

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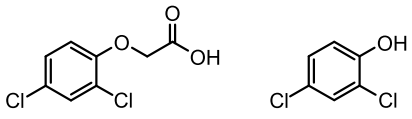
in Figure 3. From Figure 3, the toxicological interactions in mixtures to Q67 are basically additive actions, in which R1 and R2 at 0.25 h have slight antagonism at some concentration levels, whereas R2 at 12 h and R3 at 0.25 h display slight synergism at some concentration levels, which may be caused by experimental and fitting errors. It can be expected that the mixture toxicity of 2,4-D and 2,4-DCP can be predicted by the concentration addition model from the concentration−response information of single 2,4-D and 2,4-DCP.

Plots of CI_x with 95% OCIs of five mixture rays to *C. elegans* versus the percent effects (x %) are shown in Figure 4. Figure 4 shows that, except the ray R1, the combination index (CI) values of the other rays (R2, R3, R4, and R5) at 12 and 24 h are almost more than 1, which illustrates the antagonism existing in the mixtures.

DISCUSSION

Different Model Organisms. Figures S1 and S2 of the Supporting Information illustrate that different organisms have diverse responses to the same chemicals or mixture rays. For instance, the herbicides such as simetryn, bromacil, and hexazinone showed a more severe toxicity to *Chlorella pyrenoidosa* and a lower toxicity to Q67, whereas the fungicide metalaxyl possessed a high toxicity to Q67 but not to *C. pyrenoidosa*.[26] In addition, the interactions in the same binary mixtures are significantly different. In this work, the toxicological interactions in mixtures to Q67 are basically additive actions, whereas most of the mixture rays to *C. elegans* display antagonisms. The process of bioluminescence is related to luciferase.[27,28] Pesticides act as luciferase inhibitors by preventing the luciferin from entering the luciferase-active sites.[29,30] The reason why binary pesticide mixtures produce antagonism to *C. elegans* but additive actions to Q67 is at present very unclear because of the lack of the molecular mechanism of the relative mixtures, which need urgent further study.

Different Toxic Effects of Parent Pesticide and Its Metabolite. In this paper, to both tested organisms, the toxicity of metabolite 2,4-DCP is more severe than that of the parent compound 2,4-D. This result is consistent with previous reports.[31−33] It may depend on the phenoxy side chain length. Toxicity of phenoxyalkanoic acids increases with a decrease in the phenoxy side chain length.[34] Compared with 2,4-D, 2,4-DCP has a shorter phenoxy side chain length and small volume and steric hindrance (see Figure 5), which makes 2,4-DCP easily penetrate cell membranes and produce greater toxicity to organisms. However, some parent compounds to tested organisms have higher toxicity than their metabolites. The parent compound lactofen possessed higher lethality to *Daphnia magna* than its metabolites (e.g., desethyl lactofen and acifluorfen).[35] These results indicate that attention should be paid not only to the potential ecological and health risks of parent pesticide but also to those of metabolites.



2,4-D 2,4-DCP

Figure 5. Molecular structures of 2,4-D and 2,4-DCP.

Combined Toxicity of Parent Pesticide and Its Metabolite. This study would trigger attention to the interaction between parent compound and its metabolites. Generally, the joint toxicity of the parent compound and its metabolites is considered to be additive when the toxicants in the mixture behave in a similar way.[22] In this study, the combined toxicity of 2,4-D and 2,4-DCP is either antagonistic (to *C. elegans*) or additive (to Q67). However, in the study by Cáceres et al.,[22] the combined mortality toxicity of chlorpyrifos and its metabolite, 3,5,6-trichloropyridinol, to *Daphnia carinata* was additive or synergistic. These findings show that the toxicological interaction in the mixture of pesticide and its metabolite(s) is complicated and there is, at present, no uniform law of combined toxicity. Therefore, more case studies are needed.

Design of Mixtures. The toxicities of mixtures not only depend on the exposure time but also the mixture ratio and concentration level.[36] The study has come to the conclusion that to both organisms, changes in mixture ratio lead to the change of the toxicity of the mixture. In addition, different concentration levels of the same ray with the same mixture ratio have different toxicological interactions.[37,38] Conventional mixture design method, equivalent effective concentration (such as EC_{50}) ratio ray or fixed concentration ratio ray design, often only designs some mixtures with a mixture ratio and so cannot simulate the concentration distribution of various components in a real environment mixture. The EquRay (direct equipartition ray design) method developed in our laboratory[39] can rationally design many representative mixture rays with many mixture ratios with the least experiments. The toxicity test on these representative mixtures can systematically and comprehensively measure the toxicity changes of various mixtures.

Predictability of Mixture Toxicity. It is found that the pEC_{50} or pLC_{50} of various mixture rays of 2,4-D and 2,4-DCP is well correlated with the mixture ratio of 2,4-DCP ($p_{2,4\text{-DCP}}$), whether to Q67 or *C. elegans*, at short-term exposure or long-term exposure, which shows that the toxicity of any binary mixture can be predicted by the mixture ratio (see Figure 3). However, the combined toxicity or toxicological interaction is not always predictable because of the same mixture ray to different test organisms or at different exposure times or at different concentration levels having different toxicological interactions (see Figure 4). The literature by Li et al.[40] indicated that the toxicities of any mixtures having global concentration additivity (GCA) can be predicted by the concentration addition model. It is shown that the mixtures of 2,4-D and 2,4-DCP to Q67 have the GCA feature and their mixture toxicity can be predicable. In most cases, the toxicity of mixtures to *C. elegans* is antagonistic and not predictable. This unpredictable result and the predictable results from linear correlation between pLC_{50} and the mixture ratio of 2,4-DCP are contradictory, which reminds us that toxicological and chemical interactions may not be the same. Therefore, caution should be exercised in the study of the toxicological mechanism associated with the mixtures.

CONCLUSIONS

In this paper, Q67 and *C. elegans* were selected as tested organisms to evaluate the toxicities of the pesticide (2,4-D) and its metabolite (2,4-DCP) as well as their various mixtures. Only considering one organism or one exposure time, the potential ecological and health risks of 2,4-D and its metabolite

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Table 2. Chemical Abstracts Service Register Numbers (CAS RN), Purities, Molecular Weights (MW), and Stock Concentrations of Two Chemicals

chemicals	abbr.	CAS RN	purity (%)	MW (mol g^{-1})	stock (g L^{-1})
2,4-dichlorophenoxyacetic acid	2,4-D	94-75-7	99	221.0	1.567
2,4-dichlorophenol	2,4-DCP	120-83-2	99	163.0	1.292

2,4-DCP as well as their mixtures might be underestimated or even misestimated. These results remind that when evaluating the risks of pesticides, the potential of its metabolites and their mixtures should come into notice.

MATERIALS AND METHODS

Chemicals. 2,4-D and 2,4-DCP (the molecular structures shown in Figure 5) were purchased from Sigma (USA). Some physical properties and the concentration of stocks are listed in Table 2. All stock solutions were prepared in Milli-Q water with 1% dimethyl sulfoxide and stored in darkness at 4 °C before being used.

Bioluminescence Inhibition to Q67. The freeze-dried Q67 was purchased from Beijing Hamamatsu Corp., Ltd. (Beijing, China). The medium formula, culture condition, and the relative light unit (RLU) determination are performed according to the method described previously in the literature by Xu et al.[41] The toxic effect (E) of a chemical or a mixture ray to Q67 is expressed as a bioluminescence inhibition (I), calculated as follows:

$$I = \frac{aR_0 - aR}{aR_0} \times 100\% \quad (1)$$

where aR_0 is the average of the RLU of Q67 exposed to the controls (24 parallels), and aR is the average of the RLU of the test toxicant or mixture (three parallels) in one microplate. Considering the effect of time on a bioluminescence inhibition, the exposure times are set as 0.25 and 12 h, respectively.

Lethal Toxicity to *C. elegans*. Wild-type strains (N_2) of *C. elegans* and *Escherichia coli* OP50 (the food for *C. elegans*) were gained from the Institute of Medicine, Tongji University. *E. Coli* OP50 culture refers to Girard et al.[42] The *C. elegans* culture, blank and treatment group design, and lethality autoscaling are performed according to the method described previously by Tang et al.[43] The toxic effect (E) of a chemical or a mixture ray to *C. elegans* is expressed as lethality (L) of worms.[43]

$$L = \left(1 - \frac{Cen}{Ben}\right) \times 100\% \quad (2)$$

where Ben refers to the average number of worms at a certain time in the six blanks and Cen refers to the numbers of surviving *C. elegans* exposed to a chemical or a mixture ray after normalization. Considering the effect of time on lethality, the exposure times are set as 12 and 24 h, respectively.

Design of Binary Mixtures. 2,4-D with different concentrations and its metabolite, 2,4-DCP with diverse concentrations, coexisted in aquatic environment and constituted a complex binary mixture system. In order to explore the toxicity rule of the whole mixture concentration space effectively and reasonably, the EquRay method[39,44] was selected to design five concentration ratios in the binary mixture system. Five concentration ratios correspond to five mixture rays (denoted as R1, R2, R3, R4, and R5). The concentration ratios of 2,4-D and 2,4-DCP for EC_{50} (Q67) or LC_{50} (*C. elegans*) analysis are 5:1, 4:2, 3:3, 2:4, and 1:5, respectively. The concentration fractions or mixture ratios of the ith component (p_i) is defined as the ratio of the concentration of the ith component in a mixture (i = 2,4-D or 2,4-DCP) to the total concentration of the mixture.[45]

$$p_i = \frac{c_i}{c_{2,4\text{-D}} + c_{2,4\text{-DCP}}} \quad (3)$$

The values of various p_is in mixture rays and the concentrations of stocks of various rays are listed in Table S1 of the Supporting Information.

Concentration–Response Model. To describe the toxic effects (E) of various concentrations (c) quantitatively, especially at low concentrations, the concentration effect data were fitted to two nonlinear functions, Weibull (eq 4) and Logit (eq 5).[44]

$$E = 1 - \exp(-\exp(\alpha + \beta \cdot \lg(c))) \quad (4)$$

$$E = \frac{1}{1 + \exp(-\alpha + \beta \cdot \lg(c))} \quad (5)$$

where α and β are the location and shape parameters of the CRC to be estimated.

The goodness of fit is described by the fitted coefficient of determination (R^2) or RMSE. The higher the R^2 or the lower the RMSE, the better is the fit.[46] The 95% OCIs of the fitted CRC are constructed by the APTox program[44,47,48]

Toxicological Interaction Evaluation. The toxicological interaction in the mixture is qualitatively identified by the concentration addition model.[29] The CI_x[38] values were calculated to quantitatively describe the degree of toxicological interaction (eq 6).

$$CI_x = \sum_{i=1}^{m} \frac{c_i}{EC_{x,i}} \quad (6)$$

where m is the number of components in the mixture, $EC_{x,i}$ is the effect concentration of the ith component that provokes the effect of (I or L) x % when applied singly, and c_i is the concentration of the ith component in the mixture that provokes the effect of (I or L) x %.

When 1 is between the 95% OCIs, the interaction is considered as an additive action or no interaction.[37] When 1 is under the lower limit or above the upper limit of the OCIs, it is illustrated as the antagonism or synergism existing in mixtures, respectively.[38]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b02282.

Mixture ratios of 2,4-D and 2,4-DCP in mixture rays and the CRCs of 2,4-D, 2,4-DCP, and five mixture rays (R1, R2, R3, R4, and R5) to two organisms at two exposure times (PDF)

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Notes

The authors declare no competing financial interest.

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